2911-MiniSymp
Unique Structure Aspects of the Cytochrome b_6f Complex
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The 3.0 Å crystal structure of the cytochrome b_6f complex from the transformable cyanobacterium *Nostoc* sp. PCC 7120 [1] is very similar to that previously determined from the thermophilic *M. laminosus* [2, 3) and the green alga, *C. reinhardtii* [4]. Two unique structure features in the complex are: (i) heme c_n, not found in the bc_1 complex [2, 4]; (ii) the existence of two rotamer states of heme b_p, with the heme in the *Nostoc* structure rotated by 180° about the normal to the membrane plane relative to its orientation in *M. laminosus*. The rmsd between the Cα atom positions of the eight subunits of *M. laminosus* and *Nostoc* is 0.83 Å; rmsd for the *p*-side prosthetic groups, cyt *f* heme and the [2Fe-2S] cluster, are 0.66 and 0.51 Å, and hemes b_p and b_n, 5.74 and 0.28 Å [5].
Heme c_n bound covalently to the n-side of the cytochrome *b* polypeptide has an open coordination site [2, 4] that could bind plastoquinone, as shown [3], or oxygen. EPR spectroscopic analysis [6] shows that ferric heme c_n does not bind a variety of common heme ligands [7]. However, the reduced state binds NO, forming a novel heme-Fe(II)-NO species and could bind O_2. PQ analogues bind, $\leq$ 1 molecule per dimer, to the native complex at high concentrations, implying that they can replace a single PQ molecule in the binding cavity near heme c_n.
[1] Baniulis *et al.*, submitted, 2008.
[2] Kurisu *et al.*, Science, 302, 1009-, 2003.
[3] Yamashita *et al.*, J. Mol. Biol., 370, 39-, 2007.
[4] Stroebel *et al.*, Nature, 426, 413, 2003.
[5] Yamashita et al., in preparation, 2008.
[6] Zatsman *et al.*, JACS, 128: 14246, 2006.
[7] Hendrich *et al.*, in preparation, 2008. [NIH-GM-032383 (WAC), NIH-GM-077387 (MPH)].

2912-MiniSymp
Controlled Motion of the Iron-Sulfur-protein Head Domain in the bc1 Complex - Insights from Famoxadone binding to *Rh. Sph.* bc1
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The membrane protein ubiquionol cytochrome *c* oxido-reductase (cytochrome bc_1, bc_1) combines the oxidation of ubiquinol to ubiquinone with proton pumping in an ingenious way and with high efficiency, building a membrane potential for ATP synthesis. Arguably, this may be the reason why bc_1 is found in the vast majority of life forms ranging from simple bacteria, via yeast to mammals and plants. The reaction bc_1 performs is crucial, featuring both ubiquinol oxidation and ubiquinone reduction. Not surprisingly, the bc_1 complex has been the target of effective pesticides widely used in crop protection and as antibiotics against parasites. Crystallographic studies of inhibitors bound to bc_1 provide both clues for pesticide design as well as mechanistic insights into its function. Here, we report on the crystal structure of *Rh. sph.* bc_1 with famoxadone bound at the Qo site at 3.1 resolution. We compare and contrast it with the same complex from bovine mitochondrial bc_1 and with related complexes formed with stigmatellin as well as other related inhibitors. It appears that famoxadone, while assuming a slightly different conformation in *Rh. sph.* bc_1, arrests the head domain of ISP in the same way as in mitochondrial bc_1. No direct hydrogen bond forms between the inhibitor and Fe2S2 ligands as expected. However, the head domain of ISP lifts off its cyt *b* binding crater compared to the stigmatellin bound form. Furthermore, additional crystal structures of several mutants of *Rh. sph.* bc_1 provide further insight into local changes that affect the cd1/cd2 helix and the ef loop, which is implicated in the control of the motion of ISP head domain.

2913-MiniSymp
Probing membrane proteins: Proton translocation by respiratory Complex I subunits and mrp antiporters
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Respiratory Complex I (NADH:ubiquinone oxidoreductase) plays a key role in bioenergetics, coupling electron transfer to proton translocation across the mitochondrial inner membrane. Malfunction of Complex I contributes to normal aging as well as neurodegenerative diseases. Despite its key role in biology and medicine, the mechanism of energy coupling in Complex I is far from understood. This mechanism uses electron transport through the hydrophilic domain to drive proton translocation through the relatively distant membrane-bound domain. While the hydrophilic domain is structurally determined and the electron pathway is well characterized, very little is known about the membrane-bound domain, and how it performs proton translocation.
Four of the seven membrane-bound subunits are homologous to mrp antiporters, proteins that transport Na^+ and H^+ simultaneously in opposite directions across membranes. This homology makes these subunits prime candidates for harboring proton channels, and thus participating actively in Complex I function. We probe these subunits individually, monitoring their proton-pumping activity and antiporter-inhibitor sensitivity. For this purpose we have developed a new, quantitative method for monitoring proton translocation across membranes[1,2]. The method utilizes a pH-sensitive, membrane-impermeable nanoprobe[3] enclosed inside closed membrane vesicles and monitored by a semi-automatic titration and spectrometer[4], enabling robust, high-precision data from membrane-bound proton pumps (see Thom Leidings poster).
1. Gustavsson, T., Eek, M., Leiding, T., Peterson Årsköld, S., and Hägerhäll, C. (2008) Submitted to Biochimica et Biophysica Acta Bioenergetics.
2. Leiding, T., Górecki, K., Vinogradov, S., Hägerhäll, C., and Peterson Årsköld, S. (2008) Submitted to Analytical Biochemistry.
3. Finikova, O., Galkin, A., Rozhkov, V., Cordero, M., Hagerhall, C., and Vinogradov, S. (2003) J. Am. Chem. Soc. 125, 4882-4893.
4. Autonomous Science Machines TM

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2914-Plat
Nucleosome Sliding by ACF is Processive and Bidirectional
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The packaging of DNA into chromatin presents significant challenges to essential nucleic acid transactions such as transcription, replication, and repair. This challenge is overcome by a variety of chromatin remodeling enzymes, which couple the energy of ATP hydrolysis to the assembly and mobilization of nucleosomes, thereby modulating the accessibility of DNA. It is generally accepted that most chromatin remodeling complexes employ a nucleosome 'sliding' mechanism, wherein a histone octamer is translated along DNA without trans-displacement. However, it has been difficult to directly characterize structural dynamics and kinetic intermediates during the remodeling process. Here we report a single-molecule Förster resonance energy transfer (FRET) based assay to monitor in real time the remodeling of individual nucleosomes by the human ATP-utilizing chromatin assembly and remodeling factor (ACF). We demonstrate that ACF can processively slide histone octamers along the DNA in an ATP dependent manner, exhibiting multiple kinetic pauses during translocation. A predominant pause was observed after ~7 base pairs of DNA translocation, independent of the DNA sequence. Surprisingly, ACF exhibits bidirectional translocation activity and rapid switching of directionality: a single ACF functional unit can dynamically translate the histone octamer back and forth many times along the DNA prior to dissociation, suggesting that the functional unit of ACF is a dimer. These previously unknown remodeling intermediates and dynamics have significant implications on the mechanistic understanding of chromatin remodeling enzymes.

2915-Plat
A Biophysical Model Of Interactions Between Transcription Factors And Chromatin
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Binding of transcription factors (TFs) to DNA is critical for triggering a cascade of events that lead to gene expression. The role of chromatin in this process is not considered by traditional biochemical models of protein-DNA interaction, or is limited to the passive DNA sequestration by the nucleosomes. Taking into account dynamic structure of chromatin is important for understanding transcription regulation in eukaryotes.
Here we present a biophysical model of interactions between TFs and chromatinized DNA. The model takes into account dynamics of nucleosomes as well as other important features of eukaryotic regulatory regions such as the clustering TF binding sites, nucleosome-positioning DNA signals etc. Our model

demonstrates that a wide range of biological phenomena can be explained by interactions between TFs and chromatin, and provides a quantitative description of the following processes:

- cooperative binding and synergistic action of non-interacting TFs;
- access of TFs to chromatinized DNA;
- displacement of nucleosomes from regulatory regions;
- rapid evolutionary changes in arrangement and membership of TF-binding sites in eukaryotic regulatory regions.

Strikingly, we found that cooperative binding of TFs to chromatinized DNA is identical to the Monod-Wyman-Changeux model of allosteric cooperativity in hemoglobin, pointing at a general mechanism of cooperativity employed in a range of biological systems. This parallel allowed us to use classical results in biochemistry to gain deep insights into the mechanisms of gene regulation.

2916-Plat

Nucleosome Depleted Region In Promoter Improves Robustness In Gene Expression

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Nucleosomes assembled onto promoters are critical for eukaryotic transcription regulation. Recent genome wide mapping of nucleosome positioning in *Saccharomyces cerevisiae* revealed that many promoters have nucleosome depleted region (NDR) that contains the transcription factor (TF) binding sites (TFBS), presumably to provide access for the factors. However, the necessity and advantage of this configuration is not well understood. We perturbed the relative position between TFBS and nucleosomes on two G1/S cell-cycle regulated promoters, and used time-lapse fluorescence microscopy to probe their transcriptional activity in individual cells through multiple cell cycles. We find that although localization of TFBSs to NDR is not required for transcription activation, it significantly reduces variability in expression between cell cycles. TFBSs localized to NDR activate transcription in every cell cycle with high reliability. In contrast, when the same TFBSs are located within positioned nucleosomes, activation was bimodal: nearly fully active in some cell cycles, and essentially undetectable in others. Interestingly, this ''on/off'' transcription pattern displays short-term ''memory'', or epigenic inheritance, from the previous mother cell cycle. Furthermore, the probability of ''on/off'' cycles could be affected by varying the TF concentration and nucleosome acetylation level. Our results have significant implications for the function and evolution of NDR, and for the relationship between the chromatin structure on promoters and the reliability of gene expression.

2917-Plat

Identifying and quantifying load-dependence in transcription initiation by T7 RNA Polymerase

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To access the genetic code to be transcribed to RNA, RNA polymerases must first melt the DNA, opening a ''transcription bubble''. In single molecule optical tweezers studies of initiation by T7 bacteriophage RNA polymerase (T7 RNAP), we have found that the lifetime of the initiation complex is strongly dependent on tension applied to the upstream DNA, indicating, in accord with structural studies, that initiation by T7 RNAP likely involves mechanical deformation of the DNA. The tension dependence diminishes considerably in the absence of free ribonucleotides, suggesting that this mechanical deformation of the DNA template does not coincide with binding, but instead with the opening of the transcription bubble. A new maximum-likelihood method will be presented, in which data do not have to be grouped into force bins, to identify the force-dependent step or steps in initiation by T7 RNAP and to estimate the associated rates and distances to the transition state from these experiments. This method is generalizable to systems with more complicated kinetic pathways, as long as there are two observable states (e.g. bound and unbound) and an irreversible final transition.

2918-Plat

Single RNA Counting Reveals Alternative Modes Of Gene Expression In Yeast

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Proper execution of gene expression programs requires the coordination of many steps along the gene expression pathway. Preceding all downstream steps, transcriptional control is the most critical step in gene expression regulation. The transcription machinery has to ensure that the two most defining factors of mRNA expression, timing and amplitude of mRNA production, are properly controlled. How precisely the transcription machinery fulfills this task is not known. To address this question directly, we used an improved in situ hybridization approach that detects single mRNA molecules within fixed yeast cells. This approach provides a quantitative measure of mRNA abundance and transcriptional activity for endogenous genes in single cells. We show that expression levels are higher than reported by the literature and vary significantly among cells. Combining the single transcript measurements with stochastic simulations we demonstrate the existence of alternative modes of transcription that lead to different levels of mRNA variation in the cell. Housekeeping genes are expressed by single initiation events but no transcription bursts, resulting in low mRNA variability. In contrast, PDR5, a SAGA regulated gene, is expressed by transcription bursts; multiple transcripts are initiated within a short period of time followed by periods where no transcription occurs, resulting in significantly larger variation. This shows that yeast contains multiple modes of transcription allowing it to modulate its transcriptome according to specific physiological requirements.
To gain even further insight in the kinetic of gene expression, we used a live cell imaging approach that allows us to detect single mRNAs in yeast cells; at the site of transcription as well as in the cytoplasm. Analysis of the expression kinetics of different steps along the gene expression pathway will be presented. Supported by NIH.GM 57071.

2919-Plat

A Powerful Response to Viral Infection

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The key initial step in the response of the innate immune system to viral infection is the induction of the cytokine Interferon-β(IFNβ.) Recently, the cell-to-cell variability of IFNβ mRNA in human dendritic-cells has been studied. Specifically, the number of mRNAs produced by each of the two alleles in a cell in response to infection to by a non-pathogenic avian-flu virus was measured and surprisingly high levels of variability were observed. The induction is known to occur through the slow recruitment of several transcription factors to form an 'enhanceosome' assembly, over a period of hours. This variability referred to as noise was attributed to the dynamics of enhanceosome formation. Noise can arise because of the stochasticity of the chemical reactions due to the small copy numbers of molecules involved (termed ''intrinsic'') or due to fluctuations the number of transcription factors between cells (termed ''extrinsic). The experiment was able to determine the allelic origin of the mRNAs in a given cell and quantify the 'extrinsic noise' as the correlation between the number of alleles produced by each allele, thus isolating the intrinsic noise contribution. They found that a significant fraction (about half) of the total noise came from intrinsic noise.
We have analyzed the measured mRNA distribution and find that the observed long-tailed distribution can be fit by a power-law over roughly three decades at different times of observation. We find that a pulsing model in which the completed enhanceosome switches between a state in which transcription occurs at a very low or zero level and another in which transcription proceeds at a high rate can reproduce the power-law behavior. We discuss under what conditions this model produces mRNA distributions with the observed long-tailed behavior and how these distributions evolve with time.

2920-Plat

How Xenopus Embryos Complete DNA Replication Reliably: Solution to the Random-completion Problem

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DNA replication in *Xenopus* embryos starts at random positions along the genome and at random times during S phase. This spatiotemporal randomness implies fluctuations in the completion times and can lead to cell death if replication takes longer than the cell cycle time (approximately 25 min). Surprisingly, while the typical completion time (approximately 20 min) is close to the cell cycle time, replication failure occurs only about 1 in 300 times. These observations raise an interesting question, known as the ''random-completion problem'': How can *Xenopus* embryos accurately control the replication timing despite the spatiotemporal randomness?
We use a nucleation-and-growth model and extreme-value statistics to address quantitatively the random-completion problem. We first show that *Xenopus* embryos solve the problem by using a large reservoir of potential replication origins that are increasingly likely to initiate, a situation that leads to robust